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The Origin of Plant Sterols in the Skin Surface Lipids in Humans: From Diet to Plasma to Skin

ASHIM K. BHATTACHARYYA, PH.D., WILLIAM E. CONNOR, M.D., AND DON S. LIN, M.S.

Departments of Pathology and Physiology, Louisiana State University Medical Center (AKB), New Orleans, Louisiana; Department of Medicine and the Clinical Research Center, University of Oregon Health Sciences Center, (WEC, DSL) Portland, Oregon; and the Clinical Research Center, University of Iowa College of Medicine, Iowa City, Iowa, U.S.A.

To test the hypothesis that plant sterols found in the skin surface lipids of humans originated from diet after their absorption from intestine into plasma and then transferred to skin, we studied the 24-h excretion of plant sterols and cholesterol from skin and in feces in a hyperlipoproteinemic (type IIa) patient fed formula diets providing varying quantities of plant sterols (0–30 g/day) and cholesterol (0–1000 g/day). Upon feeding a sterol-free diet, the β-sitosterol excretion from the skin decreased progressively, from about 6 mg/day to 0.08 mg/day by 83 days and then completely disappeared. With addition of plant sterols (about 30 g/day) to the diet, β-sitosterol reappeared in the skin surface lipids and rose to nearly 5 mg/day by 6 weeks. With feeding of the sterol-free diet, the fecal excretion of β-sitosterol and the 2 other plant sterols decreased gradually and by week 4 disappeared completely from the feces and continued to be absent from the feces as long as the diet was free of plant sterols. The results demonstrated clearly that plant sterols which were absorbed into the plasma from the diet were excreted into the skin surface lipids after being transferred from the plasma to the skin.

In a previous study we demonstrated for the first time the excretion of β-sitosterol, campesterol, and stigmasterol, the commonly found plant sterols, through the skin of humans [1]. We suggested that these sterols in the skin surface lipids were transferred from the plasma to the skin after their absorption from the diet in small quantities from the intestine. We also suggested that the skin excretion of plant sterols as well as cholesterol constituted a second pathway of excretion of sterols from the body, fecal excretion being the primary pathway. Plant sterols in slightly higher amounts than normal subjects were also found in the skin surface lipids of two sisters with the newly described lipid storage disease "β-sitosterolemia and xanthomatosis" [2]. Nikkari, Schriebman, and Ahrens confirmed our observation in regard to the skin excretion of β-sitosterol in humans but in much smaller amounts [3]. This study was designed to test the hypothesis that plant sterols in the skin surface lipids in humans originated from the diet after being absorbed from the intestine into plasma and then transferred to the skin for excretion into skin surface lipids.

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Reprint requests to: A. K. Bhattacharyya, Ph.D., Department of Pathology, LSU Medical Center, 1901 Perdido Street, New Orleans, Louisiana 70112.

Abbreviations:
GLC: gas-liquid chromatography
TLC: thin-layer chromatography

MATERIALS AND METHODS

The overall plan of the study was to determine the 24-h excretion of sterols from the skin and the sterol balance in a patient fed different liquid-formula diets providing zero to high amounts of plant sterols and cholesterol. The patient who had been studied over a long period of time in the Clinical Research Center of the University Hospitals was a 64-year-old woman with type IIa hypercholesterolemia, xanthelasma, and mild hypertension with normal renal function. Incidentally, this patient was also the subject (#8) of our original study in 1972 [1]. The experimental protocol was explained to the patient in detail and informed consent was obtained in accordance with the policies of the Committee on Investigations Involving Human Beings.

Diets

Her diets consisted exclusively of liquid formulas in which dietary fat contributed 40% (saturated 15%, monounsaturated 18%, and polyunsaturated 7%), protein 15%, and carbohydrates 45% of the total caloric intake (about 1800 kcal a day) together with 8 g of cellulose and vitamin and mineral supplements to meet all nutritional requirements as previously published [4,5]. The sterol content of the diets and the duration of each dietary period are given in Table I. Diet A provided 40% of calories from fat from mixed sources, 1000 mg/day cholesterol from egg yolk and 485 mg/day plant sterols. Diet B was a practically sterol-free diet. Stripped lard was the primary source of fat in this diet. Actual analysis of the diet showed that it was practically devoid of plant sterols (< 1.5 mg/day) and cholesterol (< 5 mg/day). Diet C, a high plant sterol-containing but cholesterol-free diet, was identical to diet B in all respects except for the inclusion of approximately 30 g plant sterols per day (provided as Cytellin, Eli Lilly & Co., Indianapolis, Indiana). Diet D provided both cholesterol and plant sterols at high levels and was identical to diet C except for the addition of 1000 g/day cholesterol from egg yolk.

Radioactive Sterols

[22,23-^{3}H]-β-Sitosterol (New England Nuclear Corp., Boston, Massachusetts) was purified by thin-layer chromatography (TLC) on florisil plate developed with heptane:ethyl ether (55:45, v/v). The material that moved with the same R_f value as that of β-sitosterol (> 99% pure, Applied Sciences Labs., State College, Pennsylvania) was used. Gas-liquid chromatographic (GLC) analysis of this material gave a single peak.

All radioactivity counting was done by liquid scintillation spectrometry using 10 ml of scintillation mixture containing 4 g of 2,5,d-diphenyloxazole and 0.1 g of 1,4-*bis*-(2-phenyloxazolyl)-benzene per liter of toluene.

Collection of Skin Surface Lipids

The 24-h skin surface lipids were collected at intervals of 7–10 days in each of the dietary periods as described previously [1]. Briefly, the subject's body surface, excluding the head and hair, was gently wiped clean with a towel soaked in acetone after the subject had taken a warm shower without soap or shampoo. For the next 24 h, she wore specially cleaned standard hospital white cotton pajamas, long-sleeved shirts, and socks which had been previously extracted 3 times with boiling acetone. During the 24-h collection period the subject did not use any cosmetics and did not wash any part of her body except for hands. Further, the clothing was secured at wrist and ankle by rubber bands or by tucking into the socks. The head and forehead were covered with an acetone-washed gauze bandage when going to bed. On the next morning the subject's body surface was gently wiped with acetone-soaked sponges to collect the lipid material. Extreme care was taken to avoid food and fecal soiling of the clothing during the period of skin

TABLE I. *The daily excretion of plant sterols from the skin in the different dietary periods*

Diet	Duration in weeks	Time (weeks) and no. of SSL[a] collections	Plant sterols (mg/day)			
			β-sitosterol	Campesterol	Stigmasterol	Total
A. Moderate plant sterol and high cholesterol[b]	4	3 & 4 (2)[c]	5.30	0.40	0.16	5.86
B. Sterol-free	18	1–3 (3)	6.24	0.29	0.13	6.66
		4–6 (2)	5.38	0.12	0.08	5.58
		7–9 (3)	2.08	0.14	0.01	2.23
		10–12 (5)	0.16	0	0	0.16
		13–15 (1)	0	0	0	0
		16–18 (2)	0	0	0	0
C. High plant sterol	6	1–3 (3)	0.47	0	0	0.47
		4–6 (4)	3.47	0.09	0.04	3.53
D. High plant sterol and cholesterol	6	3–6 (4)	4.19	0.23	0.06	4.48

[a] Skin surface lipids.
[b] The other constituents of the diets are described in the text.
[c] All values are average of no. of collections shown in parentheses.

surface lipid collection. Three sets of clothing were used throughout the study. The lipids were extracted from the sponges and the clothing worn over the 24-h period by boiling with acetone. The acetone extract was evaporated to a small volume and was centrifuged to remove cell debris, hairs, and other insoluble material. The total lipids were then determined by weighing, dissolved in chloroform:methanol (2:1 v/v), and made up to 100 ml with the solvent. An aliquot was used for the isolation and quantitation of sterols by the combined method of TLC and GLC [1,6].

GLC was equipped with a hydrogen flame ionization detector and an automatic digital integrator. The glass column, a 120-cm U-tube, 4 mm ID, was packed with Diatoport-S, 80–100 mesh (Hewlett-Packard Co., Palo Alto, California) coated with 3.8% SE-30 (Applied Science Labs.). Temperatures of the column, detector, and flash heater were 230, 250, and 300°C, respectively. Helium was the carrier gas at a flow rate of 100 ml/min; the inlet pressure was 40 lb/in^2.

Skin Surface Lipid Radioactivity

In order to obtain further evidence in regard to the origin of β-sitosterol in the skin surface lipids, the patient was fed a dose of [22,23-^{3}H]-β-sitosterol, 50 days after the beginning of the sterol-free diet (diet B). About 85 μCi of [22,23-^{3}H]-β-sitosterol was fed in a breakfast meal prepared by dissolving the isotope in 5 g stripped lard and mixing with the formula, which provided a total of about 400 calories, 45 g carbohydrate, 15 g protein, and 18 g fat. This isotopic breakfast was also devoid of plant sterols and cholesterol. Skin surface lipids collected at intervals of time following the isotopic breakfast were analyzed for radioactivity in the unsaponifiable lipid fraction as described above.

Fecal Neutral and Acidic Steroids

Complete collection of the patient's stool was made throughout the study. The stools were combined into 7-day pools, homogenized with 1:1 water, and aliquots were analyzed for plant sterols by the combined TLC and GLC [6,7].

RESULTS

With the initiation of the sterol-free diet (diet B), β-sitosterol excretion from the skin decreased progressively and finally disappeared (Table I). The β-sitosterol excretion remained similar to that in the preceding dietary period (diet A) during the first 30 days after the beginning of the sterol-free diet. By 83 days, β-sitosterol excretion had decreased to 0.08 mg/day and then completely disappeared from the skin surface lipid of the patient and remained so as long as the diet remained free of plant sterols. Twenty days after the addition of about 30 g plant sterols per day to the patient's formula, β-sitosterol reappeared in measurable amounts in skin surface lipids. As the plant sterol-containing diet was continued, the excretion of β-sitosterol through the skin rose to nearly 5 mg/day by the end of the study. The other 2 plant sterols, namely, campesterol and stigmasterol, followed a similar pattern.

The daily total cholesterol excretion through the skin varied from 86.4–111.5 mg in the different dietary periods, which were not significantly different. The average daily cholesterol excretion was 100.7 mg and constituted 4% of the daily total skin surface lipid excretion.

The daily excretion of 2 other sterols, lathosterol and lanosterol, which are also excreted normally through the skin, ranged from 0.52–1.06 and 0.69–0.98 mg/day, respectively, and did not differ in the different dietary periods. The daily excretion of total lipids from the skin varying from 2.08–3.45 g/day also remained similar in the different dietary periods.

Twelve days after the feeding of a single dose of [22,23-^{3}H]-β-sitosterol the ^{3}H-radioactivity in the unsaponifiable lipid fraction of the skin surface lipid was measurable—309 dpm/g lipid. In contrast, 2 days after the feeding of [22,23-^{3}H]-β-sitosterol, the ^{3}H-radioactivity in the plasma was 21,247 dpm/dl. The ^{3}H-radioactivity in the skin surface lipids increased to 17,856 dpm/g lipid on day 22 and thereafter decreased to 7797 dpm/g lipid on day 34 and 747 dpm/g on day 56 after the feeding of [22,23-^{3}H]-β-sitosterol. By 78 days, no radioactivity could be detected in the unsaponifiable lipid fraction of the skin surface lipids. However, by day 34, the patient's plasma contained only traces of ^{3}H-radioactivity (Fig 1).

Fecal Excretion of Plant Sterols

With the beginning of the sterol-free diet (diet B), the fecal excretion of plant sterols decreased to 73 mg/day from an average value of 326 mg/day in the preceding dietary period (diet A) during which the daily plant sterol intake averaged 485 mg/day. By week 4 of the sterol-free diet, no plant sterols could be detected in the feces and continued to be absent from the feces as long as the diet remained free of plant sterols. Addition of 30 g/day of plant sterols to the diet produced a large daily excretion of these sterols in the feces.

DISCUSSION

The study demonstrated that the plant sterols that were absorbed from the diet into the plasma were transferred into the skin for excretion. Because there is no evidence to date that animal tissues synthesize plant sterols (only plants have this capacity) [8,9], and if β-sitosterol in the skin surface lipid is of dietary origin, then withdrawal of β-sitosterol from the diet should reduce and finally eliminate its presence in the skin surface lipids. With the addition of β-sitosterol back into the diet, the sterol should reappear in the skin surface lipids. This sequence was exactly what we found in the study. After 83 days on the plant sterol-free diet, the skin surface lipids in the patient were devoid of β-sitosterol. With the addition of β-sitosterol in the diet, the sterol reappeared in measurable amount in the skin surface lipids.

Moreover, the oral administration of [22,23-^{3}H]-β-sitosterol led to its initial recovery and ultimate disappearance from the skin surface lipids. These results also demonstrated that β-sitosterol after being absorbed from the diet into the blood in

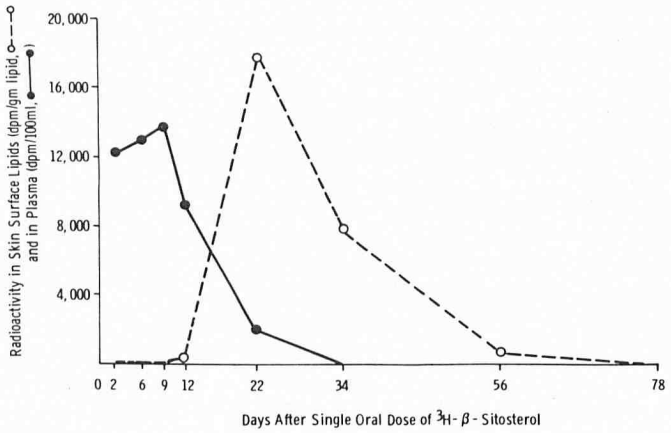

FIG 1. The radioactivity in the skin surface lipids and plasma after a single oral dose of [22,23-^{3}H]-β-sitosterol.

the gut was transferred to the skin and eventually excreted into the skin surface lipids. The ^{3}H-radioactivity in the skin surface lipids could be detected by 12 days after feeding the isotope (Fig 1) and, by mass, β-sitosterol was detected by 20 days after the addition of large amounts of plant sterols in the patient's diet (Table I). Thus, there is apparently a longer delay in appearance of β-sitosterol in the skin surface lipids because, in our previous study [1], we have observed that intravenously injected radioactive cholesterol appeared in the skin surface lipids by 7 days. The delay in appearance of β-sitosterol in the skin surface lipids, however, is in agreement with the turnover time of epidermal cells and thus suggests that plant sterols are probably incorporated only into the epidermal basal cells, whereas cholesterol is presumably incorporated into the epidermal cells during differentiation. The accumulation of β-sitosterol in the epidermal basal cells may then lead to its excretion in the skin surface lipids in association with dead cells thrown out by a holocrine mechanism.

Dietary β-sitosterol is absorbed in only small quantities by most humans [8,10] except in the syndrome of β-sitosterolemia and xanthomatosis in which plant sterol absorption is considerable [2,11]. The plasma radioactivity after the feeding of a single dose of [22,23-^{3}H]-β-sitosterol (Fig 1) did provide evidence for some absorption of β-sitosterol in our patient. However, the absorption of plant sterols must have been very small because 6 weeks of feeding very high levels (about 24 g) of β-sitosterol caused an insignificant increase in the plasma β-sitosterol concentration (0.65 mg/dl on diet C which contained a high amount of plant sterols as compared to 0.43 mg/dl on diet A containing a moderate amount of plant sterols).

With the initiation of the sterol-free diet, the fecal excretion of β-sitosterol and the 2 other plant sterols decreased gradually and remained so as long as the diet was free of plant sterols. Since β-sitosterol was barely detectable in the patient's plasma (see above) the fecal β-sitosterol during the first 3 weeks of the sterol-free diet must have been derived from the intestinal mucosa, or conceivably but unlikely from other tissues which might have sequestered β-sitosterol from the previous dietary period. In view of this point, a crucial question arises: Does the failure to recover 100% of β-sitosterol in the feces in the steady state mean that the β-sitosterol is degraded in the gut [5,12], or does it mean that the sterol is simply sequestered in the gut mucosa? The latter phenomenon would then resemble the non-ideality of Cr_2O_3 excretion because of sequestration of this unabsorbable marker in the gut [13]. This possibility in regard to β-sitosterol has not received attention before. It has been suggested that β-sitosterol is an "ideal" internal standard to correct for degradative losses by cholesterol in sterol balance studies [12]. Although the degradation of cholesterol and β-sitosterol during the intestinal transit was suggested some 15 years ago [14], to date no degradation products have been identified. In view of this, we think that it is important to recognize the above possibility in regard to β-sitosterol's use as an internal standard in sterol balance studies.

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REFERENCES

1. Bhattacharyya AK, Connor WE, Spector AA: Excretion of sterols from the skin of normal and hypercholesterolemic humans. Implications for sterol balance studies. J Clin Invest 51:2060–2070, 1972
2. Bhattacharyya AK, Connor WE: β-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J Clin Invest 53:1033–1043, 1974
3. Nikkari T, Schreibman PH, Ahrens EH Jr: In vivo studies of sterol and squalene secretion by human skin. J Lipid Res 15:536–573, 1974
4. Connor WE, Hodges RE, Blieler RE: The serum lipids in man receiving high cholesterol and cholesterol-free diets. J Clin Invest 40:894–901, 1961
5. DenBesten L, Connor WE, Kent TH, Lin DS: Effect of cellulose in the diet on the recovery of dietary plant sterols from the feces. J Lipid Res 11:341–345, 1970
6. Miettinen TA, Ahrens EH Jr, Grundy SM: Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. J Lipid Res 6:411–424, 1965
7. Grundy SM, Ahrens EH Jr, Miettinen TA: Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. J Lipid Res 6:397–410, 1965
8. Salen G, Ahrens EH Jr, Grundy SM: Metabolism of β-sitosterol in man. J Clin Invest 49:952–967, 1970
9. Nicholas HJ: The biogenesis of terpenes in plants, Biogenesis of Natural Compounds, 2nd ed. Edited by P Bernfeld. New York, Pergamon, 1967, pp 829–901
10. Gould RG, Jones RJ, LeRoy GV, Wissler RW, Taylor CB: Absorbability of β-sitosterol in humans. Metabolism 18:652–662, 1969
11. Bhattacharyya AK, Connor WE: Familial diseases with storage of sterols other than cholesterol: cerebrotendinous xanthomatosis and β-sitosterolemia and xanthomatosis, The Metabolic Basis of Inherited Disease, 4th ed. Edited by JB Stanbury, JB Wyngaarden, DS Frederickson. New York, McGraw-Hill, 1978, pp 656–669
12. Grundy SM, Ahrens EH Jr, Salen G: Dietary β-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. J Lipid Res 9:374–387, 1968
13. Davignon J, Simmons WJ, Ahrens EH Jr: Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function. J Clin Invest 47:127–138, 1968
14. Ahrens EH Jr: A review of the evidence that dependable sterol balance studies require a correction for the losses of neutral sterols that occur during intestinal transit, Atherosclerosis: Proceedings of the 2nd International Symposium. Edited by RJ Jones. New York, Springer-Verlag, 1970, pp 248–252

Correction

The dates of the *4th CIRD Symposium* announced in the February 1983 issue should have been given as October 21–23, 1983.